produce an avian cell culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype.

REMARKS

I. Status Summary

Claims 44–54 are now pending in the subject U.S. patent application and have been finally rejected. Claims 44-54 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contentions that these claims failed to comply with the written description and enablement requirements of 35 U.S.C. § 112, first paragraph. Claims 44-54 have been subjected to a series of rejections under 35 U.S.C. § 112, second paragraph, upon the contention that the claims are indefinite.

A series of rejections under 35 U.S.C. § 102 and a series of obviousness type double-patenting rejections have been presented based on the following documents: Allioli (1994, *Devel Biol*, 165:30-37); Chang (1997, *Cell Biol Intl*, 21:495-499); U.S. Patent No. 6,333,192; U.S. Patent No. 6,354,242; U.S. Patent No. 5,340,740; U.S. Patent No. 5,656,479; U.S. Patent No. 5,840,510; U.S. Patent No. 6,156,569; U.S. Patent Application Serial No. 08/446,021; U.S. Patent Application Serial No. 09/094,176; U.S. Patent Nos. 6,333,192 and 5,340,740 in view of Chang (1995, *Cell Biol. Intl*, Vol 19, pg. 143-149).

Claims 49, 50, and 51 have been amended. No new matter has been added by virtue of the claim amendments. Reconsideration of the application as amended and based on the arguments set forth below is respectfully requested.

II. Response to Objections

The United States Patent and Trademark Office (herein after the "Patent Office") has objected to the abstract upon the contention that it includes legalistic language, such as the term "comprising". This objection is addressed by the amendment of the abstract as presented herein wherein the word "including" is substituted for the word "comprising". The Examiner is invited to contact the undersigned for clarification of this amendment as necessary.

The Patent Office also objects to the specification upon the contention that line 1 of the specification needs to be updated. Applicants have updated the previously filed Preliminary Amendment to reflect that the parent application of the present application, U.S. Serial No. 09/370,708, issued as U.S. Patent No. 6,333,192 on December 25, 2001. Withdrawal of this objection is also respectfully requested.

III. Response to the First Rejection under 35 U.S.C. § 112, first paragraph

Claims 44-54 have been rejected under 35 U.S.C. § 112, first paragraph, on the contention that the claimed combination was not contemplated by applicants as of the filing date of present application and is therefore not disclosed in a manner such that one of ordinary skill in the art would realize that applicants were in possession of the invention. See Official Action, page 2, paragraph 3. In particular, the Patent Office contends that the specification did not contemplate the following aspects of the currently claimed invention: culturing avian PGCs for one or two months and culturing avian feeder cells isolated from the gonad/genital ridge with PGCs isolated from embryos later than stage 14. The Patent Office further contends that the specification does not teach the culture conditions required to maintain PGCs in culture for one or two months in the presence of avian feeder cells isolated from the gonad/genital ridge. Applicants have carefully considered this rejection and respectfully traverse the same as follows.

Claim 44 as currently pending recites a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising a preconditioned feeder matrix; conditioned media; and avian primordial germ cells and avian stromal cells collected from an avian embryo later than stage 14 (Hamburger & Hamilton, Series of Embryonic Chicken Growth, *J Morphol* 88:49-92, 1951; hereinafter "H&H") and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype. This combination is believed to be clearly described in the present U.S. patent application as filed. Moreover, also for the reasons set forth below, the present U.S. patent application is believed to be fully entitled to its August 9, 1999 priority date. For example, at page 11, lines 11 and 12, it is clearly recited that the cultures of the present invention include a feeder matrix, which can be a preconditioned feeder matrix. See page 11, lines 16-23, of the present application. The isolation of avian gonadal cells comprising primordial germ cells collected from an avian embryo is clearly recited at page 10, lines 17-21. The isolation of cells from avian embryos preferably after stage 14 is described at page 10, lines 3-7. The H&H staging system is also clearly referred to at page 10, lines 6 and 7. The isolation of embryonic gonadal PGCs and stromal cells is also described at page 10, lines 13-17. Finally, the use of conditioned medium is further described at page 13, line 9-20.

Turning now to dependent claims 45-54, the elements recited in the dependent claims find support as follows:

Claim Number	Claim Element	Support and Specification
45	Wherein the avian stromal cells and/or gonadal cells	Page 10, lines 14 and 15
46	Wherein the avian stromal cells are genital ridge cells	Page 11, lines 3-10
47	Wherein the preconditioned feeder matrix is derived from avian gonadal cells	Page 11, lines 13-15
48	Wherein the preconditioned feeder matrix is derived from avian genital ridge cells	Page 11, lines 13-15; page 19, lines 14-16
49	Wherein the avian gonadal cells are derived from avian embryo later than stage 14 (H&H)	Page 10, lines 1-9; page 11, lines 13-15
50	Wherein the avian genital cells are derived from an embryo later than stage 14 (H&H)	Page 10, lines 1-9; page 11, lines 13-15
51	Wherein the conditioned media is BRL conditioned media	Page 13, lines 9-12
52	Wherein the conditioned media further comprises a supplemental growth factor	Page 13, lines 9-20.
53	Wherein the embryonic stem cell phenotype is sustained for at least one month	Page 14, lines 4-7
54	Wherein the embryonic stem cell phenotype is sustained for at least two months	Page 14, lines 4-7

In view of the foregoing, applicants respectfully submit that claims 44-54 are fully in compliance with the written description requirement as set forth at 35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of the rejection of these claims under 35 U.S.C. § 112, first paragraph is respectfully requested. Allowance of claims 44-54 is also respectfully requested.

IV. Response to Second Rejection under 35 U.S.C. § 112, First Paragraph

Claims 44-54 are also rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification, while being enabling for a culture comprising PGCs and avian feeder cells does not reasonably provide enablement for culturing PGCs and avian feeder cells for one to two months. Official Action, paragraph 4, page 3. While claims 44-54 are rejected as a whole, the rejection appears to focus on the one to two month cultures as recited in claims 53 and 54. Applicants respectfully traverse this rejection and submit the following based upon this understanding.

Support and guidance for the claimed cultures can be found throughout the subject U.S. patent application as filed, including, but not limited to, Examples 1-4. Avian embryonic cells in accordance with the present invention can be cultured for at least one to two months as is typical for primary cell culture, as recited at page 14, lines 4-5 of the subject U.S. patent application as filed. Applicant respectfully submits that one to two month old cultures of PGCs and avian feeder cells are adequately enabled by the disclosure of the application as filed.

It appears that the Patent Office is requiring the presentation of working examples with respect to claims 53 and 54. It is well settled that the working examples are not required and indeed, claims should never be rejected upon the sole grounds that working examples are not present. Accordingly, withdrawal of the rejection of claims 53 and 54 under 35 U.S.C. § 112, first paragraph is respectfully requested.

Summarily, applicants respectfully submit that 35 U.S.C. § 112, first paragraph, requires merely that the disclosure enable the claims as presented. Applicants respectfully submit that this standard has clearly been met. Accordingly, withdrawal of the rejection of claims 44-54 under 35 U.S.C. § 112, first paragraph, is respectfully requested. Allowance of claims 44-54 is also respectfully requested.

V. Response to Rejection under 35 U.S.C. § 112, Second Paragraph

Claim 44 is rejected upon the contention that the phrase "undifferentiated avian cells expressing an embryonic stem cell phenotype" is unclear. The

specification clearly defines the ES cell phenotype as having a large nucleus, prominent nucleolus, and little cytoplasm. As the standards for definiteness are measured through the eyes of one of ordinary skill in the art, applicants respectfully submit that this definition provides all the information that is required for one of ordinary skill in the art to understand the metes and bounds of the claims. Additional functional characteristics are also provided at page 9, lines 15-23 wherein common immunological markers and the ability to differentiate extensively are described. That is, undifferentiated avian cells expressing an embryonic stem cell phenotype are pluripotent.

It is further asserted that the phrase "preconditioned feeder matrix" is unclear. The term "preconditioned" is clearly defined at page 11, line 20 of the subject U.S. patent application file wherein it states that the feeder matrix is cultured in the presence of media for a period time prior to the depositing of gonadal cells comprising primordial germ cells in contact with the feeder matrix, e.g. a time sufficient to initiate and establish production of, for example, growth factors or other factors by the feeder matrix. It is further asserted that the term "preconditioned" is a term understood by those of skill in the art, and the use of the term here is consistent with its use in the art. Applicants respectfully submit that this guidance would be sufficient for one of ordinary skill in the art to understand the metes and bounds of the use of the phrase "preconditioned feeder matrix" in the claims. Accordingly, applicants respectfully submit that the term "preconditioned feeder matrix" in the claims is definite and would be understood by one of ordinary skill in the art.

It is further asserted that the phrase "conditioned media" is indefinite. Applicants respectfully submit that the phrase "conditioned media" would be readily recognized by one of ordinary skill in the art in that it refers to media that has been conditioned by the addition of growth factors or other reagents. Indeed BRL conditioned media is a commercially available product. Applicants have amended the claims to recite that "BRL" is meant to refer to Buffalo Rat Liver. This amendment is supported by the specification in that a source of the BRL conditioned media is described in the specification at page 13, lines 12-15. Additionally, the Table of Abbreviations list BRL as an abbreviation for Buffalo Rat Liver. Accordingly, applicants respectfully submit that the recitation of the phrase "conditioned media" in the specification is definite and would be understood by one of ordinary skill in the art.

It is further asserted that the phrase "(H&H)" is indefinite, upon the contention that it is unclear whether (H&H) is intended to further limit the stage or if it is a method of staging as required in the claim. The specification has been amended to correct a typographical error, wherein "Hamburger" appears as "Hanburger". Applicants respectfully submit that, as defined in the specification

at page 10, lines 7-8, the term "(H&H)" refers to the Hamburger & Hamilton scale, which is an art-recognized staging scale. Thus, the usage of this term is meant to describe the stage of embryo from which the cells are isolated. The H&H scale is to be contrasted with the Eyal-Giladi & Kochav (hereinafter "EGK") scale, which uses Roman numerals to denote the stages, most of which are before the egg is laid. Accordingly, applicants respectfully submit that the recitation of the phrase "(H&H)" in the specification is definite and would be understood by one of ordinary skill in the art.

It is further asserted the metes and bounds of the recitation "stromal cells" is indefinite. As disclosed in the present U.S. patent application as filed, stromal cells are meant to refer to structural cells isolated from the gonadal and/or genital ridge region of an avian embryo in conjunction with the isolation of the primordial germ cells from the same regions. Applicants wish to point out that contrary to the contention of the Patent Office, stromal cells are not merely "part of an organ or other structure", but rather are more restrictively defined by the cited website as "connective tissue cells of an organ found in the loose connective tissue" (see definition of "stromal cells" at http://cancerweb.ncl.ac.uk/cgi-bin/omd?stromalcells). As such, the Patent Office's assertion that "any cell isolated from an animal is a stromal cell" is inaccurate. In accordance with the definition of "stromal cells" presented, which applicants submit is a definition one of ordinary skill in the art would attach to the term, a stromal cell is a structural cell (*i.e.* a connective tissue cell) that is isolated along with primordial germ cells when the latter are isolated from the gonadal and or genital ridge region of an avian embryo. As such, applicants respectfully submit that one of ordinary skill in the art would clearly understand what it meant by the term "stromal cell" as presently recited in the claims.

It is further asserted that the metes and bounds of a matrix "derived" from a gonadal or genital ridge cell cannot be determined. In this regard, applicants have understood this rejection to refer to claims 47 and 48, which recite that the preconditioned feeder matrix is derived from avian gonadal cells and from avian genital ridge cells respectively. Described at page 11, lines 13-15, the feeder matrix can be derived from or provided by the organ or tissue in which the primordial germ cells are located, *e.g.*, the gonad. Thus, the term "derived from" simply means that the feeder matrix is prepared using gonadal cells. Applicants respectfully submit that the usage of the term "derived from" in the context of a preconditioned feeder matrix would be understood by one of skill in the art. Therefore, applicants respectfully submit that the term "derived" is definite as used in claims 47 and 48.

It is further asserted that the metes and bounds of cells "derived" from an embryo later than stage 14 is unclear because any cell is "derived" from such

cells because all cells "are derived from gamete" and gametes "are derived from an embryo later than stage 14". It is respectfully submitted that in the context of claims 49 and 50, which refer to the avian gonadal cells and avian genital ridge cells that are derived from an embryo later than stage 14, it is meant that these are isolated from an embryo later than stage 14. Accordingly, the claims have been so amended. Support for this amendment can be found at page 4, lines 3-6 and page 10, lines 3-8. Therefore, applicants respectfully submit that amended claims 49 and 50 are definite.

It is further asserted that it is unclear how PGCs isolated from an embryo later than stage 14 are distinguished from PGCs isolated from a stage X or stage 14 embryo. Applicants respectfully note that the H&H system, which uses stage numbers in Arabic numerals, is a more precise staging system than the EGK staging system, which uses stage numbers in Roman numerals. That is, there are more divisions in the H&H staging system so that a stage later than 14 in Arabic numerals is quite different than a stage later than XIV in Roman numerals. Additionally, the EGK staging system refers to pre-gastrulation stages of embryonic development. Stage XIV (EGK) thus occurs at least 40-45 hours before stage 14 (H&H). Applicants respectfully submit that in accordance with the present invention, PGCs isolated from the embryo later than stage 14 (H&H) are capable of becoming embryonic stem cells, *i.e.*, they express an embryonic stem cell phenotype. Thus, they can be cultured to provide pluripotent cells. Thus, it is an aspect of the present invention that the primordial germ cells in the cultures are isolated from the embryo that is later than stage 14 on the H&H scale. Applicants respectfully submit that the stage refers to the embryo, not the cells, and one of ordinary skill in the art can easily distinguish a stage 14 embryo from a stage X embryo or a stage XIV embryo.

It is further asserted that the metes and bounds of the term "gonadal cells" and "genital ridge cells" cannot be determined. In the context of the present invention, it is meant to refer to the fact that these cells are isolated from the genital ridge or the gonad. See page 10, line 18 through page 11, line 10 of the present U.S. patent application as filed. It is an aspect of the present invention that a portion of avian gonadal cells comprising primordial germ cells collected from, for example, the genital ridge or gonads of an avian embryo, become undifferentiated cells expressing an embryonic stem cell phenotype. Thus, the present invention provides for convenience during the culturing of undifferentiated cells expressing an embryonic stem cell phenotype in that the primordial germ cells can be isolated in greater numbers from, for example, the genital ridge or gonads and are typically easier to isolate in view of the more advanced development of the avian embryo after stage 14. This is certainly one distinction from an avian embryo at stage X or even at stage 14 itself. Further,

as noted above, there are significant differences between the art recognized scaling system that employs Roman numerals and the art recognizing scaling system that employs Arabic numerals, *i.e.* the H&H scale.

And finally, applicants respectfully submit that one of ordinary skill in the art would understand the terms "gonadal cells" and "genital ridge cells" as they are used in the present application. For example, one of ordinary skill in the art would interpret "gonadal cells" to include those cells that can be isolated from the gonads, including gonadal stem cells and gonadal stromal cells. Similarly, "genital ridge cells" would be understood by one of ordinary skill in the art as cells that are found in the genital ridge. Applicants respectfully submit that one of ordinary skill in the art would not consider a "gonadal cell" or a "genital ridge cell" to be simply a cell with "some gonadal or genital function". Official Action at pages 6-7.

Claim 52 has been rejected as indefinite because the conditioned media of claim 44 does not comprise anything and therefore it cannot further comprise anything. This is addressed by the amendment of claim 52 to recite that the conditioned media comprises a supplemental growth factor.

It is further asserted that the metes and bounds of the term "sustained" in claims 53 and 54 is indefinite. As described in the present U.S. patent application as filed at page 14, lines 4 – 7, which state that the avian embryo cells of the present invention can be cultured for at least one or two months as is typical for a primary cell culture, which is significantly greater than the usual two week life of a primary cultures of cells from an unincubated avian embryo. Thus, applicants respectfully submit that claims 53 and 54 clearly recite that the cells can be cultured for at least one month.

In view of the foregoing, applicants respectfully submit that claims 44 through 54 are in compliance with 35 U.S.C. § 112, second paragraph. Accordingly, withdrawal of the rejection of claims 44 - 54 under 35 U.S.C. § 112, second paragraph is respectfully requested.

VI. Response To First Rejection Under 35 U.S.C. § 102(b)

Claims 44 - 54 have been rejected under 35 U.S.C. § 102(b) as anticipated by Allioli *et al.*, *Devel. Biol.*, 165:30-37 (1994), hereinafter "Allioli". Allioli is asserted to teach the isolation of chicken cells from gonad of a stage 27 to 28 embryo and culturing the cells in media. It is further asserted that the sample contained PGCs as well as fibroblasts that created a feeder layer in culture. It is further asserted that the PGCs of Allioli have an ES cell phenotype because both PGCs and ES cells are germ cells. It is further asserted that Allioli teaches adding steel factor, LIF and FGF to the culture. Applicants have

carefully considered this rejection and the basis for this rejection, and respectfully traverse the same as follows.

It is well settled that for a reference to anticipate a claim under 35 U.S.C. § 102(b), the reference must disclose each and every element of the claim. Applicants respectfully disagree with the Patent Office's characterization of PGCs and ES cells as equivalent because both PGCs and ES cells are germ cells. In contrast to the Patent Office's assertion, ES cells are not germ cells, as germ cells are defined as the reproductive cells in multicellular organisms (see definition at http://cancerweb.ncl.ac.uk/cgi-bin/omd?germcells). ES cells are in fact not reproductive cells at all, as that term is normally understood. ES cells are isolated from a stage of embryonic development (a blastula) that occurs before any cells have differentiated into a cell type that would plausibly be understood as a reproductive cell. Furthermore, an aspect of the present invention is to provide pluripotent or totipotent undifferentiated cells expressing an embryonic stem cell phenotype from primordial germ cells. Thus, the undifferentiated avian cells expressing an embryonic stem cell phenotype recited in claim 44 have pluripotent or totipotent characteristics, whereas primordial germ cells normally develop only into the gametes of the bird. Accordingly, applicants respectfully submit that the recitation presented in claim 44 is not anticipated by the teachings of the Allioli reference.

Claims 45 – 54 ultimately depended upon patentably distinguished claim 44. Accordingly 45 – 55 are believed to be patentably distinguished from the Allioli reference in view of this distinction. Withdrawal of this rejection is respectfully requested. Allowance of claims 45 – 54 is also respectfully requested.

VII. Response to Rejection of Claims 44 – 54 under 35 U.S.C. § 102(b) based upon Chang

The Patent Office has rejected claims 44 – 54 under 35 U.S.C. § 102(b) as being anticipated by Chang, *Cell Biol. Intl* 21:495-499 (1997) for the reasons set forth at paragraph 7, of pages 9 and 10 of the Official Action. Applicants have carefully considered this rejection and respectfully traverse the same as follows.

Applicants initially wish to point out that the Patent Office appears to be basing this rejection on the disclosures of at least two references: the Chang 1997 reference cited above, as well as a reference cited within Chang 1997, namely Chang *et al.*, *Cell Biol Intl* 19: 143-149 (1995). Applicants respectfully submit that a 102(b) rejection should not properly be based upon more than one reference. Accordingly, applicants request that this rejection under 102(b) of claims 44-54 be withdrawn and the claims be allowed at this time.

In an abundance of caution, however, applicants wish to address the bases of the Patent Office's contentions, and do so by submitting the following remarks. Claim 44 recites that the PGCs are isolated from an embryo later than stage 14. The Patent Office asserts that this element does not bear patentable weight because it does not distinguish the structure or function of the PGCs within the culture for those taught by the Chang journal article. Applicants respectfully note that prior to the invention of the present application, it was not perceived to be possible to provide a sustained culture of avian cells expressing a pluripotent embryonic stem cell phenotype that could be derived from PGCs isolated from a later than stage 14 embryo. Indeed it was believed that PGCs isolated from later than stage 14 embryos were destined for terminal differentiation into germ cells instead of other tissues.

Moreover, PGCs are more easily isolated from later stages. Additionally, applicants respectfully submit that PGCs isolated from a later than stage 14 (H&H) embryo are distinguishable from PGCs from the blood of day 2 (stage 13-14) embryos because, by definition, stage 13-14 is not "later than stage 14". Accordingly, the PGCs isolated from embryos later than stage 14 are believed to be patentably distinguishable from the disclosure of the Chang reference(s). Accordingly, a withdrawal of the rejection of claim 44 based on Chang is respectfully requested.

Applicants respectfully submit that the Patent Office's characterization of the teachings of Chang that PGCs isolated from stage 27 – 28 embryos have an ES cell phenotype because they are germ cells is not accepted in the art. As mentioned above, ES cells are not germ cells. Additionally, an ES cell phenotype as recited in the subject application and in claim 44 pertains to a pluripotent cell that is capable of differentiation into any tissue, including, but not limited to germ cells. Accordingly, claim 44 is believed to be further distinguished from the teachings of the Chang journal article in that claim 44 particularly recites that the cultured cells express an embryonic stem cell phenotype, including the pluripotent nature of the embryonic stem cell phenotype.

Summarily, claim 44 is believed to be patentably distinguished over the teachings of the Chang journal article. Claims 45 – 54 are ultimately dependent on patentably distinguished claim 44. Accordingly, withdrawal of the rejection of claims 45 – 54 under 35 U.S.C. § 102(b) based upon the Chang journal article is respectfully requested. Allowance of claims 44 – 54 is also respectfully requested.

VIII. Response to Rejection under 35 U.S.C. § 102(e) based on U.S. Patent No. 6,333,192

The Patent Office has rejected claims 44 – 54 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,333,192 to Petitte *et al.*, hereinafter the Petitte '192 patent. Applicants respectfully note that this patent issued from the application that is the immediate parent of the present application, U.S. Patent Application Serial No. 09/370,708. The present application clearly claims priority to U.S. Patent Application Serial No. 09/370,708 filed August 9, 1999. Accordingly, given that the present application and U.S. Patent Application Serial No. 09/370,708 that matured into the Petitte '192 patent have the same priority date, it is believed that a rejection of the claims under 35 U.S.C. § 102(e) is improper. Accordingly, withdrawal of this rejection of claims 44 – 54 is respectfully requested.

IX. Response to Rejection under 35 U.S.C. § 102(e) based upon U.S. Patent No. 6,354,242

The Patent Office has rejected claims 44 – 54 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,354,242 to Pardue *et al.*, hereinafter the Pardue '242 patent. See paragraph 9, page 10 of the Official Action. It is respectfully noted that the effective date of the Pardue '242 patent for purposes of 35 U.S.C. § 102(e) is March 23, 2000. Claims 44 – 54 are believed to be entitled to the priority of the parent application of the subject application, *i.e.*, August 9, 1999. Accordingly, this earlier priority date is believed to preclude application of the Pardue '102 patent against the present application under 35 U.S.C. § 102(e) and withdrawal of this rejection is respectfully requested. Allowance of claims 44 – 54 is also respectfully requested.

X. Response to Rejection under 35 U.S.C. § 102(e) based upon U.S. Patents to Petitte *et al.*

Claims 44 – 54 have been further rejected as being anticipated by U.S. Patent No. 5,340,740; U.S. Patent No. 5,656,479; or U.S. Patent No. 5,830,510, all to Petitte *et al.* (hereinafter the cited Petitte patents). This rejection is respectfully traversed. Initially, it is noted that the cited Petitte patents use a different staging for the embryos, *i.e.*, the EGK staging system instead of the H&H staging system recited in claim 44. Additionally, although the cited Petitte patents do disclose undifferentiated avian cells that express an embryonic stem cell phenotype, the specific isolation of PGCs later than stage 14 is not recited. As noted above, prior to the disclosure of the present invention, it was not believed that PGCs later than stage 14 (H&H scale) could be used to culture pluripotent undifferentiated avian cells expressing an embryonic stem cell

phenotype. Accordingly, claim 44 is believed to be patentably distinguished over the disclosure of the cited Petitte *et al.* patents.

Claims 45 – 54 are ultimately dependant on patentably distinguished claim 44. Claims 45 – 54 are therefore also believed to be patentably distinguished over the cited Petitte patents. Accordingly, withdrawal of the rejection of claims 45 – 54 is respectfully requested. Allowance of claims 45 – 54 is also respectfully requested.

XI. Response to Rejection under 35 U.S.C. § 102(e) based upon U.S. Patent No. 6,156,569.

The Patent Office has rejected claims 44 – 54 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,156,569 to Ponce de Leon *et al.* (hereinafter the Ponce de Leon '569 patent). See Official Action, page 11, paragraph 11. According to the Patent Office, the Ponce de Leon '569 patent teaches:

> [I]solating PGCs isolated from cells of stage XIV embryos. The cells were cultured with complete medium, LIF, FGF, IGF and SCF for at least 25 days (citation omitted). The PGCs were capable of creating a chimeric chicken which is a phenotype of ES cells. PGCs isolated from stage XIV are equivalent to PGCs isolated later than stage 14 as claimed because PGCs isolated from stage XIV and XIV have the same structure and function.

Official Action at page 11.

The Patent Office asserts that "avian PGCs collected from an avian embryo 'later than stage 14' as claimed does not bear patentable weight because it does not distinguish the structure or function of the PGCs within the culture from those taught by Ponce de Leon". Id. This rejection is respectfully traversed.

Applicants initially wish to point out that the Ponce de Leon '569 patent discloses the use of stage XIV PCGs, and as such, is believed to differ substantially from PGCs isolated later than stage 14. Stage XIV occurs in the first few hours before gastrulation.

Furthermore, applicants respectfully submit that the isolation of PGCs from a later than stage 14 embryo is believed to be patentably distinguished in the present invention. Prior to the disclosure of the present invention it was believed that it was not possible to obtain pluripotent embryonic stem cells from avian primordial germ cells from an embryo later than stage 14. This, coupled with the ease of isolation provided by using later embryos, is believed to patentably distinguish claim 44 over the disclosure of the Ponce de Leon '569 patent. Accordingly, withdrawal of the rejection of claim 44 based on the Ponc de Leon '569 patent is respectfully requested and allowance of claim 44 is also respectfully requested.

Claims 45 – 54 believed to be distinguished from the Ponce de Leon '569 patent in view of their dependency of patentably distinguished claim 44. Withdrawal of the rejection of claims 45 – 54 based upon the Ponce de Leon '569 patent is respectfully requested. Allowance of claims 45 – 54 is also respectfully requested.

XII. Response to First Obviousness-Type Double Patenting Rejection

Claims 44 – 54 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 2, 3, 13, 19, and 23 of co-pending U.S. patent application Serial No. 08/446,021 (hereinafter "the '021 application"). See paragraph 12, page 12 of the Official Action. This rejection is respectfully traversed.

It is respectfully submitted that claim 44 recites the isolation of primordial germ cells from an avian embryo later than stage 14 (H&H) and culturing the same to form a culture of undifferentiated avian cells expressing an embryonic stem cell phenotype. This is believed to be in marked contrast to the disclosure of somatic tissue-specific stem cells in the '021 application. Accordingly, for the reasons set forth hereinabove and incorporated here by reference, claim 44 is believed to patentably distinguished over the disclosure of the '021 application. Accordingly, withdrawal of the rejection of claim 44 is respectfully requested.

Claims 45 – 54 are believed to be patentably distinguished over the cited U.S. patent application in view of their dependency of patentably distinguished claim 44. Accordingly, withdrawal of the provisional obviousness type double-patenting rejection of claims 45 – 54 is respectfully requested. Allowance of claims 44 – 54 is also respectfully requested.

XIII. Response to Second Obviousness-Type Double Patenting Rejection

Claims 44 – 54 have been subjected to a second provision obviousness-type double patenting rejection based upon claims 21 – 27 of co-pending U.S. patent application Serial No. 09/094,176. See paragraph 13, pages 12 and 13 of the Official Action. This rejection is respectfully traversed. Applicants respectfully submit that the specific recitation of the use of primordial germ cells isolated from an avian embryo later than stage 14 in the culture as claimed in claim 44 is not disclosed in the claims 21 – 27 of the '176 application. Rather, these claims are directed to a method of introducing a DNA sequence into an *in ovo* embryo using a cell expressing an embryonic stem cell phenotype. But the unexpected characteristic of the presently claimed culture, namely that primordial germ cells isolated from an avian embryo later than stage 14 (H&H) can be cultured as pluripotent embryonic stem cells, is believed to patentably distinguish

current claim 44. Accordingly, withdrawal of this rejection of claim 44 is respectfully requested.

Claims 45 – 54 are ultimately dependent on patentably distinguished claim 44 and are believed to be distinguished from the disclosure of noted claims of the '176 application based on this dependency. Accordingly, withdrawal of the rejection of claims 45 – 54 based on cited claims of the '176 application is respectfully requested. Allowance of claims 45 – 54 is also respectfully requested.

XIV. Response to Third Obviousness-Type Double Patenting Rejection

The Patent Office has rejected claims 44 – 54 under the judicially created doctrine of obviousness-type double patenting being unpatentable over claims 1, 19 and 35 of U.S. Patent No. 6,333,192 in view of the Chang journal article. See page 13, paragraph 14 of the Official Action. Applicants submit herewith a Terminal Disclaimer that addresses the subject application in light of U.S. Patent No. 6,333,192. Accordingly, with the timely filing of a Terminal Disclaimer herewith, it is respectfully requested that this rejection be withdrawn.

XV. Response to Fourth Obviousness-Type Double Patenting Rejection

Claims 44 – 54 have been subjected to an obviousness-type double patenting rejection based upon claims 1 and 8-10 of the Petitte '740 patent in view of the Chang journal article. See paragraph 15, pages 13 and 14 of the Official Action. This rejection is respectfully traversed. While claims 1 and 8-10 of the subject application do claim a sustained culture of undifferentiated avian cells having an ES cell phenotype and methods of making such culture, the proposed combination of these claims and the Chang reference do not disclose the use of primordial germ cells isolated from an avian embryo later than stage 14 to form a culture comprising undifferentiated avian cells expressing an embryonic stem cell phenotype, as recited in claim 44. Accordingly, the proposed combination does not disclose each and every element of the claimed invention. Therefore, it is respectfully submitted that a *prima facie* case of obviousness is not supported by the proposed combination. Accordingly, withdrawal of this rejection of claim 44 is respectfully requested. Allowance of claim 44 is also respectfully requested.

Claims 45 – 54 are dependent upon patentably distinguished claim 44. Claims 45 –54 are believed to be patentably distinguished from the proposed combination based upon this dependency. Accordingly, withdrawal of this rejection of claims 45 –54 is respectfully requested, and allowance of claims 45 – 54 is also respectfully requested.

XVI. Response to Fifth Obviousness-Type Double Patenting Rejection

Claims 44 – 54 have been rejected under the doctrine of obviousness-type double patenting as being unpatentable over claim 1 of the Petitte *et al.* '470 patent or claim 1 of the Petitte *et al.* '510 patent in view of the Chang journal article. The proposed combination of claim 1 the Petitte *et al.* '470 patent or the Petitte *et al.* '510 patent and the Chang journal article do not disclose each and every element of currently pending claim 44. Particularly, there is no disclosure of the use of primordial germ cells isolated from an embryo after stage 14 in the culture of sustained undifferentiated avian cells expressing a pluripotent embryonic stem cell phenotype. Accordingly, a *prima facie* case of the obviousness of claim 44 is not believed to be established. Withdrawal of this rejection is respectfully requested. Allowance of claim 44 is also respectfully requested.

Claims 45 – 54 are dependent upon patentably distinguished claim 44. Claims 45 –54 are believed to be patentably distinguished from the proposed combination based upon this dependency. Accordingly, withdrawal of this rejection of claims 45 –54 is respectfully requested, and allowance of claims 45 – 54 is also respectfully requested.

XVII. Response to Sixth Obviousness-Type Double Patenting Rejection

Claims 44 – 54 have been subjected to an obviousness-type double patenting rejection over claims 1-12 of the Ponce de Leon '569 patent in view of the Chang journal article. Applicants respectfully note that the Ponce de Leon '569 patent is not commonly owned with the subject application and thus an obviousness-type double patenting rejection is not believed to be appropriate. Applicants now elect to respond to the rejection upon the assumption that a rejection under 35 U.S.C. § 103 was intended.

The proposed combination of the Ponce de Leon '569 patent and the Chang journal article do not disclose each and every element of the present claim 44. Particularly, there is no disclosure of a sustained culture of undifferentiated avian cells expressing a pluripotent embryonic stem cell phenotype using PGCs isolated from a later than stage 14 embryo in the culture. Accordingly, applicants respectfully submit that a *prima facie* case of the obviousness of claim 44 has not been made. Withdrawal of this rejection of claim 44 is therefore respectfully requested. Allowance of claim 44 is also respectfully requested.

Claims 45 – 54 are dependent upon patentably distinguished claim 44. Claims 45 –54 are believed to be patentably distinguished from the proposed combination based upon this dependency. Accordingly, withdrawal of this

rejection of claims 45 –54 is respectfully requested, and allowance of claims 45 – 54 is also respectfully requested.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Pursuant to 37 C.F.R. § 1.121, attached hereto is a marked-up version of the changes made to the specification, claims, and Abstract by the current amendment. The attached pages are captioned "**Version with Markings to Show Changes Made**". On those pages, words to be deleted are indicated in brackets, and words to be added are underlined and in bold text.

CONCLUSION

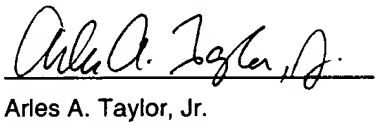
In light of the above Amendments and Remarks it is respectfully submitted that the present application is now in proper condition for allowance, and such action is earnestly solicited.

If any minor issues should remain outstanding after the Examiner has had an opportunity to study the Amendment and Remarks, it is respectfully requested that the Examiner telephone the undersigned attorney so that all such matters may be resolved and the application placed in condition for allowance without the necessity for another Action and/or Amendment.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiencies of payment or credit any overpayments associated with the filing of this Amendment After Final to Deposit Account No. **50-0426**.

Respectfully submitted,
JENKINS & WILSON, P.A.

Date: 11/27/2002 By: 

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Enclosures

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

At Page 1, beginning at line 3, please insert the following heading and paragraph:

Cross Reference to Related Applications

This application is a Continuation of prior U.S. Patent Application Serial No. 09/370,708 filed August 9, 1999, which is now U.S. Patent No. 6,333,192, the contents of which are herein are incorporated by reference.

The line at page 1, line 19 was amended as follows:

H&H - [Hanburger] **Hamburger** & Hamilton staging system

The paragraph beginning at page 10, line 1, was amended as follows:

Avian embryos from which cells are obtained for carrying out the present invention are preferably in a stage after the formation of the primitive streak and are preferably in a stage prior to sexual differentiation. Avian embryos from which cells are obtained for carrying out the present invention are preferably after stage 14, more preferably stage 14 to stage 45, even more preferably stage 15 to stage 31, including stages 17, 18, 19, 20 and 21, and most preferably in stage 27 to 30 of development on the [Hanburger] **Hamburger** & Hamilton (H&H) staging system.

IN THE CLAIMS:

Claim 49 has been amended as follows:

49. (Once Amended) The sustained culture of claim 47 wherein the avian gonadal cells are [derived] **isolated** from an avian embryo later than stage 14 (H&H).

Claim 50 has been amended as follows:

50. (Once Amended) The sustained culture of claim 48 wherein the avian genital ridge cells are [derived] **isolated** from an embryo later than stage 14 (H&H).

Claim 51 has been amended as follows:

51. (Once Amended) The sustained culture of claim 44 wherein the conditioned media is **Buffal Rat Liver (**BRL**)** conditioned media.

52. (Once Amended) The sustained culture of claim 44 wherein the condition media [is further comprised of] **comprises** a supplemental growth factor selected from the group consisting of leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), fibroblast growth factor (FGF), basic fibroblast growth factor (bFGF), stromal cell factor (SCF), steel factor (SF), transforming growth factor-β1 (TGF-β1), and anti-retinoic acid.

IN THE ABSTRACT

A method of producing undifferentiated avian cells expressing an embryonic stem cell phenotype. The method includes the steps of collecting avian gonadal cells [comprising] **including** primordial germ cells from an avian embryo after the formation of the primitive streak; depositing the avian gonadal cells in contact with a preconditioned feeder matrix; and growing the avian gonadal cells on the pre-conditioned feeder matrix in the presence of media for a time sufficient to produce an avian cell culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype.